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(21) International Application Number: PCT/CA94/00317 (22) International Filing Date: 8 June 1994 (08.06.94) (30) Priority Data: 073,378 9 June 1993 (09.06.93) US (60) Parent Application or Grant (63) Related by Continuation US 073,378 (CIP) Filed on 9 June 1993 (09.06.93) (71) Applicant (for all designated States except US): CONNAUGHT LABORATORIES LIMITED [CA/CA]; 1755 Steeles Av- enue West, Willowdale, Ontario M2R 3T4 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): SIA, Charles, D., Y. [GB/CA]; 27 Mabley Crescent, Thornhill, Ontario L4J 2Z7 (CA). CHONG, Pele [CA/CA]; 32 Estoril Street, Richmond Hill, Ontario L4C 0BC (CA). KLEIN, Michel, H. [CA/CA]; 16 Mundo Boulevard, Willowdale, Ontario M2P 1B9 (CA). (74) Agent: STEWART, Michael, I.; Sim & McBurney, Suite 701, 330 University Avenue, Toronto, Ontario M5G 1R7 (CA).		(81) Designated States: AU, BR, CA, CN, FI, JP, KR, NO, NZ, RU, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.	
(54) Title: TANDEM SYNTHETIC HIV-1 PEPTIDES <div style="text-align: center;"> <p>CLTB-34(MAP)</p> <p>—p24E— —CLTB-55—</p> <p>GPKEPFRDYVDRFYKRRKRIHIGPGRAFYITKN > k</p> <p>GPKEPFRDYVDRFYKRRKRIHIGPGRAFYITKN > k</p> <p>GPKEPFRDYVDRFYKRRKRIHIGPGRAFYITKN > k</p> <p>GPKEPFRDYVDRFYKRRKRIHIGPGRAFYITKN > k</p> </div> <div style="text-align: center;"> <p>CLTB-36(MAP)</p> <p>GPKEPFRDYVDRFYKNKRKRIHIGPGRAFYTTKN > k</p> <p>GPKEPFRDYVDRFYKNKRKRIHIGPGRAFYTTKN > k</p> <p>GPKEPFRDYVDRFYKNKRKRIHIGPGRAFYTTKN > k</p> <p>GPKEPFRDYVDRFYKNKRKRIHIGPGRAFYTTKN > k</p> </div> <div style="text-align: center;"> <p>CLTB-56</p> <p>GPKEPFRDYVDRFYKRRKRIHIGPGRAFYITKN > k</p> <p>GPKEPFRDYVDRFYKRRKRIHIGPGRAFYITKN > k</p> <p>GPKEPFRDYVDRFYKRRKRIHIGPGRAFYITKN > k</p> <p>GPKEPFRDYVDRFYKRRKRIHIGPGRAFYITKN > k</p> </div> <div style="text-align: center;"> <p>P24E-V3MN(MAP)</p> <p>GPKEPFRDYVDRFYKRIHIGPGRAFYTTKN > k</p> <p>GPKEPFRDYVDRFYKRIHIGPGRAFYTTKN > k</p> <p>GPKEPFRDYVDRFYKRIHIGPGRAFYTTKN > k</p> <p>GPKEPFRDYVDRFYKRIHIGPGRAFYTTKN > k</p> </div> <div style="text-align: center;"> <p>—V3MN—</p> <p>VP-T-8(MAP)</p> <p>—V3(BRU)— —V3(MN)—</p> <p>GPKEPFRDYVDRFYKNTRKSIRIQRGPGRAFYTTKN > k</p> <p>GPKEPFRDYVDRFYKNTRKSIRIQRGPGRAFYTTKN > k</p> <p>GPKEPFRDYVDRFYKNTRKSIRIQRGPGRAFYTTKN > k</p> <p>GPKEPFRDYVDRFYKNTRKSIRIQRGPGRAFYTTKN > k</p> </div> <div style="text-align: center;"> <p>—VP—</p> <p>CLTB-91(MAP)</p> <p>KQIINMWQEEVEKAMYANKRRIHIGPGRAFYTTKN > k</p> <p>KQIINMWQEEVEKAMYANKRRIHIGPGRAFYTTKN > k</p> <p>KQIINMWQEEVEKAMYANKRRIHIGPGRAFYTTKN > k</p> <p>KQIINMWQEEVEKAMYANKRRIHIGPGRAFYTTKN > k</p> </div>			

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TITLE OF INVENTION
TANDEM SYNTHETIC HIV-1 PEPTIDES

REFERENCE TO RELATED APPLICATION

5 This application is a continuation-in-part of United States patent application Serial No. 08/073,378 filed June 9, 1993.

FIELD OF INVENTION

10 The present invention relates to the field of immunology, and, in particular, is concerned with synthetic peptides containing T- and B-cell epitopes from human immunodeficiency virus proteins.

BACKGROUND TO THE INVENTION

15 AIDS is a disease which is the ultimate result of infection with human immunodeficiency virus (HIV). Currently, there is no effective vaccine which can protect the human population from HIV infection, so the development of an efficacious HIV-vaccine is urgently required. Previously, HIV-1 particles exhaustively
20 inactivated by chemical treatments, a vaccinia vector encoding the whole envelope protein (gp160) of HIV-1, and purified recombinant gp120 have been evaluated as candidate HIV vaccines. Although inactivated HIV-1 virus preparations elicited a T-cell-mediated Delayed-Type
25 Hypersensitivity (DTH) reaction in humans, and vaccinia/gp160 and gp120 recombinant vaccine candidates induced virus neutralizing antibodies, none of these immunogens has been shown to be an efficacious human HIV vaccine (ref. 1 - the literature references referred to
30 herein are listed at the end of the specification).

 The inventors' interest in HIV vaccinology is to develop synthetic HIV-1 peptides for incorporation into vaccines and consider that the vaccinia HIV-1-recombinant subunit used in conjunction with these HIV-1 peptide
35 vaccines may lead to the elicitation of more effective immune responses against HIV-1. To design synthetic HIV vaccine candidates, immunogenic viral B-cell

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neutralization epitopes (BE) containing a high degree of conserved sequence between viral isolates are linked to functional T-helper cell determinant(s) (THD) to elicit a strong and long lasting cross-protective antibody response. In addition, HIV-specific cytotoxic T-lymphocyte (CTL) epitopes may be included in the synthetic constructions to elicit cell-mediated immunity to HIV infection.

A specific and preferential spatial relationship between certain T- and B-cell epitopes may be necessary for tandem epitopes to be efficiently processed and thereby rendered immunogenic. Thus, it is important to identify the appropriate T- and B-cell epitope sequences in HIV-1 proteins and assemble them in the optimal configuration so that both T- and B- cell memory can be elicited effectively and antibodies of the desired specificity produced. THDs have been found not to be universal and are immunologically functional only when presented in association with the appropriate Major Histocompatibility Complex (MHC) class II antigens. There is a characteristic hierarchy of T-cell epitope dominance. To develop an effective synthetic AIDS vaccine, it is therefore important to utilize the most potent THD of the various HIV-1 gp160, gag, pol and other gene products. Recent studies have indicated that the gag gene products may play a crucial role in eliciting an immune response against HIV infection. Thus, clinical progression of AIDS is associated with a reduction of circulatory antibodies to the gag p24 protein and antibodies raised against an immunodominant gag p17 peptide are capable of inhibiting HIV-1 infection in vitro (refs. 2, 3).

In our published International Patent Application WO 90/13564, there are described the identification and characterization of a T-cell epitope of the core protein, p24E of HIV-1 and the construction of synthetic chimeric

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peptides comprising the amino acid sequence of the T-cell epitope linked to an amino acid sequence of a B-cell epitope of an envelope or core protein of HIV-1. By linking the B-cell epitopes to the T-cell epitopes, an immune response to the B-cell epitope was induced, whereas no such response was observed when the B-cell epitope was not so linked. Data is presented in such published application with respect to the p24 B-cell epitope, BE3 epitope, ENV epitope and V3A epitope, all derived from the HIV-1/LAV isolate, with and without linker sequences between the epitopes.

Specific constructs which are tested in the published WO specification are BE3 linked to the C-terminal end of p24E by direct coupling or to the N-terminal end of the p24E either by two proline residues or by direct coupling, ENV linked to the N-terminal end and linked to the C-terminal end of p24E in both cases by two proline residues, and V3A linked to the N-terminal end of p24 by two proline residues.

The V3A sequence tested in that publication (residues 308-327) of the variable loop of HIV-1 gp120 from HIV-1/LAV isolate was made immunogenic by linking the molecule to the N-terminus of p24E with a proline-proline linker.

It is known from U.S. Patent No. 4,925,784 (Crowl) to provide by recombinant means a fusion protein comprising amino acids 15 to 512 from the gag protein and 44 to 140 of the env protein of the LAV isolate of HIV-I (HTLV-III), i.e., a polypeptide or protein containing 1093 amino acids, considerably longer than any synthetic peptide, which do not exceed 150 amino acids in length and generally are not more than 50 amino acids long. Such large molecule fusion proteins are described as being useful in diagnostic applications and vaccine materials.

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The envelope glycoprotein (env) of human immunodeficiency virus (HIV) is highly variable between independent isolates and also sequential isolates from a single infected individual. The amino acid variability in env is concentrated into specific variable regions (mostly in the surface portion gp120 generated by the proteolytic maturation of the initial gp160 gene product), with other regions being less variable. However, the most variable regions often contain neutralizing epitopes so that the virus partially evades the host's immune response and establishes a persistent infection. This variability presents problems for diagnostic techniques based upon specific interactions, with separate or mixed reagents usually being employed to test samples for HIV-1. This variability also presents problems for any possible vaccine or immune therapy, since any suitable agent will have to give a response towards the many strains of HIV-1.

Thus, in generating an immune response in a host to a plurality of immunologically distinct HIV isolates, two problems exist. Firstly, any particular host in an outbred population will have a particular HLA haplotype and will thus differentially respond to a particular T-cell epitope. Secondly, antibodies may not recognize or neutralize a plurality of immunologically distinct HIV isolates and in particular HIV isolates that have been freshly harvested from patients as primary field isolates.

It would be advantageous to provide for the purposes of diagnosis, generation of immunological reagents, treatment and vaccination against HIV, synthetic peptides comprising T-cell epitopes to which a plurality of hosts will respond and B-cell epitopes from protein of different HIV isolates including primary field isolates.

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SUMMARY OF THE INVENTION

The present invention is directed towards the provision of synthetic peptides, specifically synthetic HIV-1 peptides, useful for mounting an immune response against infection by HIV or for detecting HIV infection, wherein the synthetic HIV-1 peptides comprise a T-helper determinant (T-cell epitope) of the HIV-1 core protein, particularly p24E of amino acid sequence GPKEPFRDYVDRFYK (SEQ ID NO: 2), and amino acid sequences corresponding to B-cell epitopes from HIV-1 proteins, specifically gp160, gag and pol proteins, vaccines against AIDS comprising at least one of such synthetic HIV-1 peptides and compositions, procedures and diagnostic kits for detecting HIV antigens using such synthetic HIV-1 peptides.

By the term "Synthetic Peptide" as used herein, there is meant the joining of a T-cell epitope containing amino acid sequence to a B-cell epitope containing amino acid sequence to form a synthetic T-B or B-T construct, using, for example, a peptide synthesis process, such as described in Example 1 below.

The prevalent HIV-1 strain found in the AIDS population of North America and Western Europe belongs to the HIV-1(MN) isolate. A synthetic HIV vaccine capable of protecting against this serotype, therefore, may contain p24E as THD and the neutralization epitopes of the HIV-1(MN) proteins as B-cell epitopes. Two regions or epitope clusters in the extracellular component of the HIV-1(MN) envelope protein, gp120, have been shown to elicit neutralizing antibodies against the virus. One of these regions is the third hypervariable (V3) loop encompassing the amino acid residues 301 to 335 of the gp120(MN) (Reference 4). Strain and group-specific monoclonal antibodies isolated from individuals infected with the MN isolate were shown to recognize different

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core amino acid sequences at the crown region of the V3 loop (Reference 5).

The other epitope cluster of gp120 that elicits neutralizing antibodies is the CD4 binding site. Studies with monoclonal antibodies isolated from HIV-1 infected individuals and chimpanzees have indicated that the neutralization epitopes in the CD4 binding site are formed by noncontiguous amino acid residues from multiple sites of gp120.

Moreover, results on these two types of neutralizing antibodies have shown that the in vitro neutralization of a given dose of HIV-1 virus may be achieved by a much lower concentration of V3-specific neutralizing monoclonal antibody than of one reacting against the CD4 binding site.

In the construction of synthetic peptides of the present invention, the inventors have chemically synthesized a panel of linear synthetic HIV-1(MN) peptides (shown in Table I below - the Tables appear at the end of the descriptive text) containing different flanking sequences adjacent to the highly conserved sequence (GPGR - SEQ ID NO: 1) at the crown region of the V3(MN) loop, linked either to the amino (N-) or carboxy (C-) terminus of the THD, p24E (GPKEPFRDYVDRFYK - SEQ ID NO: 2). In addition, the inventors have synthesized additional panels of linear synthetic peptides (as shown in Tables VI, VII, IX, X and XI below).

In addition, five tetrameric peptides as depicted in Figure 1 in which B-cell epitope containing sequences were linked to the C-terminus of p24E, have been prepared and investigated, namely p24E-V3MN(MAP) containing the linear p24E-V3MN sequence; CLTB34(MAP), containing the linear CLTB-34 sequence; CLTB-36(MAP), containing the linear CLTB-36 sequence; CLTB-91(MAP), containing the linear CLTB-91 sequence; and VP-T-B(MAP), containing the

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linear VP sequence (see Figure 1); each VP sequence comprising a hybrid V3 sequence of the residues 307 to 316 and 315 to 325 of HIV-1(MN) and HIV-1(BRU) isolates, respectively, linked to the C-terminus of p24E.

5 In accordance with one aspect of the present invention, there is provided a synthetic peptide, which comprises at least one amino acid sequence comprising a T-cell epitope of the gag protein of a human immunodeficiency virus (HIV) isolate linked at the N-
10 terminal or C-terminal end thereof, to at least one amino acid sequence comprising a B-cell epitope of the V3 loop of the envelope protein of an HIV isolate, wherein, when located at said N-terminal end, the B-cell epitope containing sequence and the T-cell epitope containing
15 sequence are directly coupled. Such synthetic peptides are novel and not disclosed in the aforementioned WO 90/13564.

In accordance with another aspect of the present invention, there is provided a synthetic peptide, which
20 comprises at least one amino acid sequence comprising a T-cell epitope of the gag protein of a human immunodeficiency virus (HIV) isolate linked at the N-terminal or C-terminal end thereof, to at least one amino acid sequence comprising a B-cell epitope of the gp41
25 protein of an HIV isolate comprising the sequence X_1LKDWX_2 , wherein X_1 is E, A, G or Q and X_2 is A or T, particularly ELKDWA, (see reference 10) or a sequence capable of eliciting an HIV specific antiserum and recognizing the sequence X_1LKDWX_2 . Such synthetic peptides are novel and
30 not disclosed in the aforementioned WO 90/13564.

A further aspect of the invention provides the synthetic peptide molecule, comprising a plurality of individual chimeric synthetic peptides linked to form a multimeric molecule, each said individual synthetic
35 peptide comprising an amino acid comprising a T-cell epitope of a gag or envelope protein of a human

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immunodeficiency virus (HIV) isolate linked to an amino acid sequence comprising a B-cell epitope of a gag or envelope protein of an HIV isolate. Such multimeric molecules are novel and not disclosed in the
5 aforementioned WO 90/13564.

The invention further comprises antibodies specific to any of the synthetic peptides provided herein and nucleic acid sequences coding for a synthetic peptide as provided herein, which nucleic acid sequences may be
10 incorporated into an expression vector.

The HIV isolate with which the present invention is concerned generally is an HIV-1 isolate. The amino acid sequences of the synthetic peptides comprising the sequences of the T-cell and B-cell epitope containing
15 sequences may be those of a variety of HIV-1 isolates, including LAV, BRU, MN, SF2, RF, PRI, 1714, 2054, HXB2, Z6, BX08, IIB and SC. Consensus sequences of different isolates also may be employed.

In the embodiment of the invention where the B-cell epitope-containing amino acid sequence is from the V3 loop protein, the amino acid sequence preferably comprises the sequence GX_1GX_2 , where X_1 is P or Z and X_2 is R, K or Q or a sequence capable of eliciting an HIV-specific antiserum and recognizing the sequence GX_1GX_2 ,
20 particularly the sequence GPGR. The B-cell epitope containing sequence may comprise a B-cell epitope containing V3 loop sequences from at least two different HIV-1 isolates and may comprise a consensus sequence of the V3 loop of at least two HIV-1 primary isolates.
25

In the various embodiments of the invention, the T-cell epitope containing amino acid sequence preferably comprises the sequence of a p24 protein, for example P24E, P24N, P24L, P24M and P24H, particularly P24E. The sequences of those peptides, which are highly conserved
30 among HIV-1 isolates, are given below in Tables I and IX. Such sequences also include a portion, variation or
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mutant of any of the selected sequences which retains the T-cell properties of the selected sequence.

The amino acid sequence comprising the B-cell epitope may be directly coupled to the C-terminal amino acid of the amino acid sequence comprising the T-cell epitope.

The B-cell epitope containing sequence may be additionally linked to a further amino acid sequence containing an HIV T-cell epitope, which may be that of a gag or envelope protein of HIV. The B-cell epitope containing sequence also may be linked to a further amino acid sequence containing a B-cell epitope of HIV. B-cell epitopes of the gp41 protein and containing the X₁LKDWX₂ sequence may be joined one to another or with amino acid sequences containing the B-cell epitope of the V3 loop.

The multimeric molecules provided herein may comprise a plurality of identical individual chimeric synthetic peptides and preferably comprise the synthetic peptides defined above.

The present invention further provides an immunogenic composition, comprising an immunoeffective amount of at least one synthetic peptide provided in accordance with the invention or at least one nucleic acid molecule encoding any one of the synthetic peptides, and a pharmaceutically-acceptable carrier therefor. In addition, the present invention provides a method of immunizing a host, preferably a human host, comprising administering thereto an immunogenic composition as provided herein.

The immunogenic composition may comprise a plurality of ones of the synthetic peptides selected to provide an immune response to a plurality of immunologically-distinct HIV-1 isolates and preferably further selected to provide the immune response in a plurality of hosts differentially responsive to any particular T-cell epitope.

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A particularly useful "cocktail" of peptides useful in the immunogenic composition comprises the peptides identified as CTLB-36, CTLB-91 and BX08 in the Tables below. This composition may further contain the peptide
5 identified on MPK-2 in Table XI below.

The immunogenic composition may be formulated for mucosal or parenteral administration. The immunogenic composition may further comprise at least one other immunogenic or immunostimulating material, particularly
10 an adjuvant, such as aluminum phosphate or aluminum hydroxide.

The present invention also extends to diagnostic kits useful for detecting HIV specific antibodies in a test sample, the kit comprising:

- 15 (a) a surface;
- (b) at least one peptide having an amino acid sequence epitopically specific for the HIV-specific antibodies immobilized on the surface and as provided herein;
- 20 (c) means for contacting the antibodies and the at least one immobilized peptide to form a complex; and
- (d) means for detecting the complex.

In a further aspect of the invention, there is provided a diagnostic kit for detecting HIV antigen in a
25 test sample, the kit comprising:

- (a) a surface;
- (b) an antibody epitopically specific and non-cross-reactive for distinct epitopes of the HIV antigen immobilized on the surface and raised to the
30 peptides provided herein;
- (c) means for contacting the antibodies and the HIV antigen to form a complex; and
- (d) means for detecting the complex.

BRIEF DESCRIPTION OF DRAWING

35 Figure 1 shows the construction of tetrameric peptides which are capable of eliciting polyclonal

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antibody responses in mice and/or guinea pigs against HIV-1;

Figure 2 contains a graphical representation of antibody responses in guinea pigs immunized with non-infectious, non-replicating HIV-1 (IIIB)-like particles followed by boosting with an HIV-1 peptide cocktail, as provided in an embodiment hereof; and

Figure 3 contains a graphical representation of the reactivity of guinea pig antisera raised after priming with non-infectious, non-replicating HIV-1 (IIIB)-like particles and boosted with an HIV-1 peptide cocktail, as provided in an embodiment hereof.

GENERAL DESCRIPTION OF INVENTION

In one embodiment, the present invention comprises peptides having amino acid sequence corresponding to antigenic determinants of the V3 loop linked to the N- or C-terminus of the highly conserved T-cell epitope, p24E, of HIV-1 core protein, p24 (as shown in Table I). These peptides can have, for example, the sequence **RIHIGPGRAFYTTKNGPKEPFRDYVDRFYK** (V3MN-p24E - SEQ ID NO: 10) and **GPKEPFRDYVDRFYKRIHIGPGRAFYTTKN** (p24E-V3MN - SEQ ID NO: 9) corresponding to the amino acids 311-325 of the V3 (MN) loop printed in bold face (throughout this specification bolded sequences are the B-cell epitope-containing amino acid sequences, unless otherwise noted) linked to the N- and C-terminus of p24E (amino acids 291-305 of the HIV-1(MN) core protein, p24) as T-cell epitope containing amino acid sequences, respectively. These peptides also can have, for example, the sequence **RKRIHIGPGRAFGPKEPFRDYVDRFYK** (CLTB-32 - SEQ ID NO: 13) and **GPKEPFRDYVDRFYKRKRIHIGPGRAF** (CLTB-28 - SEQ ID NO: 12) corresponding to the amino acids 309-320 of the V3(MN) loop printed in bold face linked to the N- and C-terminus of p24E, respectively. These peptides also can have the sequences **RKRIHIGPGRAFYTTKNGPKEPFRDYVDRFYK** (CLTB-35 - SEQ ID NO: 7) and **GPKEPFRDYVDRFYKRKRIHIGPGRAFYTTKN** (CLTB-34 -

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SEQ ID NO: 6) corresponding to the amino acids 309-325 of the V3(MN) loop printed in bold face linked to the N- and C-terminus of p24E, respectively. The peptides can also have the sequence **NKRKRIHIGPGRAFYTTKNGPKEPFRDYVDRFYK** (CLTB-37 - SEQ ID NO: 4) and **GPKEPFRDYVDRFYKNKRKRIHIGPGRAFYTTKN** (CLTB-36 - SEQ ID NO: 3) corresponding to the amino acids 307-325 of the V3 (MN) loop printed in bold face linked to the N- and C-terminus of p24E, respectively.

10 These peptides are capable of eliciting polyclonal HIV-specific antibody responses in mice, guinea pigs and monkeys (Tables III and IV).

 In another embodiment, the present invention comprises multimeric molecules such as the tetrameric
 15 peptides (as disclosed in Figure 1) capable of eliciting polyclonal antibody responses against HIV-1 in mice or guinea pigs (Table XIII). These multimeric molecules, can have, for example, four linear p24E-V3MN sequences (SEQ ID NO: 9) tetramerized using lysine branching
 20 peptide synthesis technology, hence designated p24E-V3MN(MAP - multi-antigenic peptide). These tetramers can also contain, for example, four lysine-branched CLTB-34 sequences (SEQ ID NO: 6), hence designated CLTB-34(MAP). These tetramers can also contain, for example, four
 25 lysine-branched CLTB-36 sequences (SEQ ID NO: 3), hence designated CLTB-36 (MAP). These tetramers also may contain, for example, four lysine-branched CLTB-91 sequences (SEQ ID NO: 20) and hence designated CLTB-91(MAP). These tetramers also can contain, for example,
 30 four lysine-branched VP-T-B linear sequence, **GPKEPFRDYVDRFYKNTRKSIRIQRGPGRAFYTTKN** (SEQ ID NO: 15) comprising a hybrid V3 sequence, VP(**NTRKSIRIQRGPGRAFYTTKN** - SEQ ID NO: 16), linked to the C-terminus of p24E (SEQ ID NO: 2). The hybrid V3 sequence, VP, itself comprises
 35 amino acids 307-316 (**NTRKSIRIQR** - SEQ ID NO: 17) of the V3(BRU) loop printed in bold face linked via its C-

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terminal end to the N-terminal end of the amino acid sequence 315-325 (GPGRAFYTTKN - SEQ ID NO: 18) of the V3(MN) loop shown in bold face.

The novel immunogenic compositions of the present invention comprise peptides containing immunogenic T- and B-cell epitopes of HIV, prepared as peptides which link specific antigenic determinants from the extracellular envelope domain, gp120, gp41 and the core protein p24, of HIV-1. These compositions are useful for immunization to elicit HIV-specific humoral immune responses when administered to mammals as demonstrated in mice, guinea pigs and monkeys as seen by the data presented below in the Examples.

Synthesis of peptides

To design a synthetic peptide-based HIV immunogen, linear peptides containing sequences from the V3 loop and gp41 linked to either the N- or C-terminus of peptides containing T-cell epitopes were chemically synthesized using an automated ABI 430A solid-phase peptide synthesizer, as described in Example 1. Different combinations were formulated in Freund's adjuvant (FA) or aluminum phosphate (alum) to compare their ability to induce HIV-specific immune responses in mammals.

Five multimeric molecules, designated p24E-V3MN(MAP), CLTB34(MAP), CLTB-36(MAP), CLTB-91(MAP) and T-B-VP(MAP), formed by tetramerization using lysine branching peptide synthesis technology of the respective linear tandem epitopes, i.e. p24E-V3MN, CLTB-34, CLTB-36, CLTB-91 and VP-T-B also were prepared. Their ability to elicit HIV specific immune responses in mammals when administered in alum or FA was investigated.

Immunogenicity of the linear HIV peptides in mammals

The ability of the linear HIV peptides to elicit antibody responses in mammals was examined by immunizing mice, guinea pigs or monkeys with individual peptides emulsified in FA or adsorbed to alum. After four

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injections of 100 µg each by the subcutaneous route, IgG antibody responses were determined by peptide-specific EIA and by an in-vitro syncytia-blocking assay (Tables II, III, IV, V, XII).

5 The four different V3(MN) peptides, namely V3MN, CLTB-29, CLTB-55 and CLTB-56, containing the different sequences flanking the crown portion (GPGR) of the V3 (MN) loop but lacking the p24E sequence were either non-immunogenic or poorly immunogenic irrespective of whether
10 they were injected in FA or aluminium phosphate (alum) (see Table III below). The carrier function of p24E to enhance the immunogenicity of these peptides was shown by studies performed with the respective synthetic HIV-1 peptides comprising a T-cell epitope and a B-cell
15 epitope. Thus, the synthetic HIV-1 peptides in the T-B orientation elicited V3(MN)-specific antibody responses of much greater magnitude than the respective free V3 (MN) peptides or B-T counterparts (Table III). The results of these studies, therefore, showed that the orientation of
20 the V3(MN) peptide with respect to p24E influenced the immunogenicity of the synthetic HIV-1 peptides of the present invention. A comparison of the respective anti-V3 peptide antibody titres measured in the murine and guinea pig antisera generated against the individual
25 synthetic HIV-1 peptides, administered in either FA or aluminium phosphate (alum), revealed that CLTB-36 was the most immunogenic peptide in both guinea pigs and mice (Table III).

30 The immunogenicity of CLTB-36 was further demonstrated by the ability of this peptide when formulated in the adjuvant ISA 51 to elicit a strong peptide-specific antibody response in primates (Table IV) which antibodies were virus neutralizing. Significantly, both the murine and guinea pig antisera raised against
35 CLTB-34 and CLTB-36 were able to cross-react against the

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V3 peptides of a variety of HIV-1 serotypes (Table V below).

The novel usage of p24E and a V3 sequence for the construction of immunogenic T-B peptides is further illustrated by the studies performed with three other chimeric peptides designated p24E-SP10(A), CLTB-84 and T1-SP10(A)-MN (Table I). The results in Table III below showed that when administered in either FA or alum, p24E-SP10(A) with the sequence GPKEPFRDYVDRFYKCTRPNYNKRKRRIHIGPGRAFYTTK (SEQ ID NO: 19), comprising the N-terminal end (CTRPNYNKRKRRIHIGPGRAFYTTK - SEQ ID NO: 20) of the V3(MN) loop linked to the C-terminus of p24E was able to induce good titres of CLTB-56-specific antibodies in guinea pigs. p24E-SP10(A) formulated in alum similarly elicited a high anti-CLTB-56 antibody response in Balb/c (H-2^d). In contrast, T1-SP10(A)-MN, with the sequence KQIINMWQEVEKAMYACTRPNYNKRKRRIHIGPGRAFYTTK - SEQ ID NO: 21), containing the same N-terminal end of the V3(MN) loop linked to the C-terminus of a T-cell epitope, T1 (KQIINMWQEVEKAMYA - SEQ ID NO: 22) reported in the literature (Ref. 4) was found to be poorly immunogenic in guinea pigs and Balb/c (H-2^d) mice. These data suggested that p24E served as a more effective carrier for the N-terminal sequence of the V3(MN) loop than T1. Moreover, T1 was found to mediate the enhancement of immunogenicity of CLTB-56. This result was shown by the high CLTB-56-specific antibody titres measured in the serum samples of guinea pigs immunized with the CLTB-91 peptide of the sequence, KQIINMWQEVEKAMYANKRKRRIHIGPGRAFYTTKN - SEQ ID NO: 23), comprising of CLTB-56 linked to the T-cell epitope T1 in FA or alum, and mice injected with CLTB-91 in alum. Since CLTB-91 differs from T1-SP10(A)-MN only in the V3(MN) sequences linked to the C-terminus of T1, these data show that CLTB-56 is a better B-cell epitope than the N-terminal end of V3 (MN) loop used in T1-

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SP10(A)-MN. Furthermore, CLTB-84 containing the CLTB-56 sequence linked to the C-terminus of another T-cell epitope, P24M comprising the sequence GHKARVLAEMSQVT (SEQ ID NO: 30) of p24, when administered in alum was also
5 found to be highly immunogenic in guinea pigs (Table III).

Five other panels of HIV-1 synthetic peptides were produced. In the first panel of peptides shown in Table VI (SEQ ID NOS: 38 to 47), the V3 sequences of two
10 different U.S. clinical HIV isolates, 1714 (NTRKRIHMGPGRAFYATGDIIG - SEQ ID NO: 48) and 2054 (NTRKGIHIGPGRAFYTG EIVGDIRQ - SEQ ID NO: 49), were linked to the C-terminus of p24E and T1 to form the p24E-1714 and T1-2054 respectively. In addition, the V3 consensus
15 sequence, PRI (NTRKSIPIGPGRAFYTTG - SEQ ID NO: 50), of the consensus of New York and Amsterdam isolates was linked to either p24E, T1 or p24M to form the respective T-B peptides of CLTB-PRI, T1-PRI and p24M-PRI. Furthermore, three other T-B peptides were constructed by linking p24E
20 to the V3 sequences of LAI (NTRKSIRIQRGPGRAFYTTIG - SEQ ID NO: 51), RF (NTRKSITKGPGRVIIYATGQIIIG - SEQ ID NO: 52) and a hybrid V3 sequence of MN and RF (NKRKRIHIGPGRVIIYATGQIIIG - SEQ ID NO: 53) to form the respective CLTB-V3B, CLTB-V3RF and CLTB-HB constructs.

25 A second panel of constructs are shown in Table VII (SEQ ID NOS: 54 to 68). The particular gp41 sequences used for their constructions share the neutralization epitope, ELDKWA (SEQ ID NO: 69), described in reference 6. The results in Table VIII showed that each of these
30 peptides was recognized by the human neutralizing monoclonal antibody, 2F5 (Reference 6).

Table IX shows the third panel of peptides which were constructed (SEQ ID NOS: 70 to 84). The constructions involved the use of the CLTB-56 sequence
35 for linking to either the N- or C-terminus of three different T-cell epitopes from gag namely P24N

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(QMREPRGSDIAGTTSTL - SEQ ID NO: 70), P24L
 (EEMMTACQGVGGPGHK - SEQ ID NO: 73) and P24M
 (GHKARVLAEAMSQVT - SEQ ID NO: 76) to form the B-T or T-B
 synthetic peptides respectively. Furthermore, the
 5 consensus sequence, PRI (NTRKSIPIGPGRFYTTG - SEQ ID NO:
 80) of the New York and Amsterdam isolates was also
 linked to either the N- or C-terminus of the T-cell
 epitope, P24H (PIVQNIQGQMVHQAI - SEQ ID NO: 79) or T5
 (VKYKVVKIEPLGVAP - SEQ ID NO: 82) to form the respective
 10 B-T or T-B peptides.

The fourth panel of peptides made are shown in Table
 X (SEQ ID NOS: 85 to 92). Peptides CLTB-102 and CLTB-105
 were constructs containing a hybrid sequence of gp41
 (ELLELDKWASLWNWF - SEQ ID NO: 93) and CLTB-56 linked to
 15 the C- and N-terminus of the T-helper epitope, p24E,
 respectively. CLTB-103 and CLTB-107 are peptides
 containing the CLTB-56 and the same gp41 sequence linked
 to the C- and N-terminus of P24E, respectively. For
 constructs CLTB-160 and CLTB-161, the V3 sequences from
 20 a consensus (LIP) of the London, India and Paris
 isolates, the V3 sequence (THAI) of a Thailand (HIV-1
 virus and a consensus of the primary isolates found in
 New York and Amsterdam were used to link to the C-terminus
 of the T-cell epitope, p24E and T1, respectively.

25 The fifth panel of peptides constructed is shown in
 Table XI (SEQ ID NOS: 94 to 97). The peptide, MPK-1,
 contains a copy of the gp41 neutralization epitope
 (ELDKWAS - SEQ ID NO: 98) linked via a GPG linker
 sequence at its N- and C-terminus to the T1 and p24E T-
 30 cell epitopes respectively. The construction of the
 peptide, MPK-2, was the same as MPK-1 except that the
 orientation of T1 and P24E were reversed. The
 constructions of MPK-3 and MPK-4 involved the use of two
 copies of the gp41 sequence ELDKWAS for making MPK-1 to
 35 link its N- or C-terminus to either T-1 and p24E, or p24E
 and T1, respectively.

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Immunogenicity of HIV-1 peptide cocktail

The immunogenicity of a cocktail containing five peptides of the present invention was assessed in guinea pigs (Table II). These tandem peptides consisted of:

5 CLTB-70, containing the V3 sequence of the HIV-1(SF2) isolate linked to the C-terminus of p24E; CLTB-72, containing the V3 sequence of HIV-1(IIIB) linked to the C-terminus of p24E; CLTB-74, containing the V3 sequence of HIV-1(RF) linked to the C-terminus of p24E; CLTB-76,

10 containing the V3 sequence of HIV-1(Z6) linked to the C-terminus of p24E and p24E-GP41C, containing a gp41 sequence of HIV-1(BRU) linked to the C-terminus of p24E. The results shown in Table II show that animals immunized with the cocktail formulated in FA or alum elicited high

15 antibody responses against each of the five tandem peptides.

Therefore, the above-described results show the ability of the peptide cocktail when adsorbed to alum to elicit strong antibody responses against the V3 loops of

20 four different HIV-1 isolates (SF2, IIIB, RF and Z6) and a gp41 sequence of HIV-1(BRU).

An immunization schedule using another HIV-1 peptide cocktail consisting of CLTB-36, CLTB-91, CLTB-84 (shown in Table 1) and CLTB-70 (shown in Table II) and an HIV-1

25 self-assembled, non-replicating, non-infectious HIV-like particle (as described in WO 91/058564 published May 2, 1991) were also investigated. Results depicted in Figure 2 show that guinea pigs previously immunized with the HIV-like particle emulsified in incomplete Freund's

30 adjuvant and boosted with the cocktail adsorbed to alum were found to elicit strong antibody responses against the CLTB-56 peptide. The virus neutralizing titre of the antisera against the MN isolate following the second booster injection with the cocktail was 1,091. Very

35 significantly, the results depicted in Figure 3 further show that the antisera collected from the animals post-

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second boost with the peptide cocktail demonstrated strong cross-reactivity against peptides containing V3 sequences of several laboratory grown viruses and primary clinical isolates.

- 5 Other peptide cocktails may comprise immunoeffective amounts of any of the disclosed peptides including a synthetic peptide, which comprises at least one amino acid sequence comprising a T-cell epitope of the gag protein of a human immunodeficiency virus (HIV) isolate
10 linked at the N-terminal or C-terminal end thereof, to at least one amino acid sequence comprising a B-cell epitope of the gp41 protein of an HIV isolate comprising the sequence X_1LKDWX_2 , wherein X_1 is E, A, G or Q and X_2 is A or T or a sequence capable of eliciting an HIV-specific
15 antiserum and recognizing the sequence X_1LKDWX_2 , and in particular peptide MPK-2 (SEQ ID NO. 95, Table XI).

- The functional activity of the antisera generated against the synthetic HIV-1 peptides was investigated by testing their ability to inhibit syncytia formation
20 induced by the homologous HIV-1(MN) isolate. The murine antisera following immunization with the four V3 (MN) peptides containing only the B-cell epitope containing sequences, namely V3MN, CLTB-29, CLTB-55 and CLTB-56 administered in either FA or aluminium phosphate (alum),
25 lacked syncytia-blocking activity (see Table XII below). Guinea pig antisera generated against CLTB-56 formulated in FA, but not aluminium phosphate (alum), were found to exhibit >90% syncytia inhibitory activity at a dilution of 1 in 10. The murine and guinea pig antisera raised
30 against the B-T tandem synthetic peptides namely, V3MN-p24E, CLTB-32 and CLTB-35, in either FA or alum, which showed poor antibody titres reactive against the respective B-cell epitope containing peptides namely, V3MN, CLTB-29 and CLTB-55 were also found to lack
35 syncytia-blocking activity. However, guinea pig antisera generated against CLTB-37 administered in FA or aluminium

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phosphate (alum), which contained a CLTB-56-specific antibody titre of 1 in 1,250 and 1 in 450, respectively, were both found to have syncytia-inhibition titres of 1 in 10. The results of the functional studies carried out with the antisera raised against the immunogenic T-B synthetic peptides revealed that both murine and guinea pig antisera raised against the T-B synthetic peptide, CLTB-36, in either FA or aluminium phosphate (alum) strongly inhibited syncytia-formation induced by the homologous (MN) virus. In addition, the antisera raised against CLTB-36 formulated in ISA 51 in cynomolgus monkeys were also found to have good neutralizing titres (278 and 430 in the two animals) against the MN isolate (see Table IV). The murine and guinea pig antisera generated against p24E-V3MN and CLTB-34 in FA containing higher titres of V3MN- and CLTB-55-specific antibodies than the respective antisera raised against the peptide in alum also were found to have effective syncytia--blocking activity. It was also observed that the animal species used for immunization affected the production of V3(MN)-specific functional antibodies. This effect was illustrated, for example, by the fact that, although the T-B tandem synthetic peptide CLTB-28 in FA induced the same titre of anti-CLTB-29 antibodies in mice and guinea pigs, only the antiserum raised in the latter had a high titre of syncytia-inhibition activity.

Immunogenicity of multimeric molecules in mammals

The ability of the multimeric molecules CLTB-36(MAP), CLTB-91(MAP), CLTB-34(MAP), p24E-V3MN (MAP) and VP-TB(MAP), (with their respective configurations illustrated in Figure 1) to elicit antibody responses in mammals was examined by immunizing mice and guinea pigs with the molecules emulsified in FA or alum. After four doses each of 100 µg, IgG antibody responses were determined by peptide-specific ELISA and by an in vitro syncytia-blocking assay.

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The results of the immunogenicity studies performed with the multimeric molecules in mice and/or guinea pigs are shown in Table XIII below. High titres of CLTB-56-specific peptide antibodies were generated in both mice and guinea pigs immunized with the tetramer CLTB-36 (MAP) in either FA or alum. CLTB-91 (MAP) formulated in either FA or alum similarly induced high CLTB-56-specific antibody titres in these animals. The tetrameric T-B tandem synthetic peptide CLTB-34 (MAP), p24E-V3MN (MAP) or VPTB (MAP), administered in FA also were capable of eliciting high titres of the respective CLTB-55, V3MN and VP-specific antibodies in guinea pigs. The murine and guinea pig antisera raised against CLTB-36 (MAP) in either FA or alum strongly inhibited syncytia formation induced by the HIV-1 (MN) virus (Table XIV below). The guinea pig antisera raised against the branched peptides CLTB-34 (MAP) and VP-T-B (MAP) in FA similarly exhibited potent syncytia-blocking activity.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis, treatment of HIV infections, and the generation of immunological reagents. A further non-limiting discussion of such uses is further presented below.

Vaccine Preparation and Use

It has been shown that a peptide in accordance with the invention can elicit an immune response. One possible use of the molecule is therefore as the basis of a potential vaccine against AIDS and AIDS related conditions. In a further aspect, the invention thus provides a vaccine against AIDS and AIDS related conditions, comprising a molecule in accordance with the invention.

Immunogenic compositions, suitable to be used as vaccines, may be prepared from immunogenic peptides as

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disclosed herein. The immunogenic composition elicits an immune response which produces antibodies that are opsonizing or antiviral. Should the vaccinated subject be challenged by HIV, the antibodies bind to the virus and thereby inactivate it.

Vaccines containing peptides are generally well known in the art, as exemplified by U.S. Patents 4,601,903; 4,599,231; 4,599,230; and 4,596,792. Vaccines may be prepared as injectables, as liquid solutions or emulsions. The peptides may be mixed with pharmaceutically-acceptable excipients which are compatible with the peptides. Excipients may include water, saline, dextrose, glycerol, ethanol, and combinations thereof. The vaccine may further contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness of the vaccines. Methods of achieving adjuvant effect for the vaccine include the use of agents such as aluminum hydroxide or phosphate (alum), commonly used as 0.05 to 0.1 percent solution in phosphate buffered saline. Vaccines may be administered parenterally, by injection subcutaneously or intramuscularly. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkalene glycols or triglycerides. Oral formulations may include normally employed incipients such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10-95% of the peptides.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as is therapeutically effective, protective and immunogenic.

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The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize antibodies, and to produce a cell-mediated immune response. Precise
5 amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms of the peptides. Suitable regimes for initial
10 administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations, for example, at least one pre-peptide immunization with a self-assembled, non-infectious, non-replicating HIV-like particle, such as
15 described in WO 91/058564, assigned to the assignee hereof, followed by at least one secondary immunization with the peptides provided herein. The dosage of the vaccine may also depend on the route of administration and will vary according to the size of the host.

20 Nucleic acid molecules encoding the peptides of the present invention may also be used directly for immunization by administration of the nucleic acid molecules directly, for example by injection, or by first constructing a live vector, such as *Salmonella*, BCG,
25 adenovirus, poxvirus, vaccinia or poliovirus, and administering the vector. A discussion of some live vectors that have been used to carry heterologous antigens to the immune system are discussed in, for example, O'Hagan 1992, (ref. 10). Processes for the
30 direct injection of DNA into test subjects for genetic immunization are described in, for example, Ulmer et al, 1993, (ref. 11).

The use of the peptides provided herein in-vivo may require their modification since the peptides themselves
35 may not have a sufficiently long serum and/or tissue half-life. For this purpose, the molecule of the

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invention may optionally be linked to a carrier molecule, possibly via chemical groups of amino acids of the conserved sequence or via additional amino acids added at the C- or N- terminus. Many suitable linkages are known, e.g., using the side chains of Tyr residues. Suitable carriers include, e.g., keyhole limpet hemocyanin (KLH), serum albumin, purified protein derivative of tuberculin (PPD), ovalbumin, non-protein carriers and many others.

In addition, it may be advantageous to modify the peptides in order to impose a conformational restraint upon it. This might be useful, for example, to mimic a naturally-occurring conformation of the peptide in the context of the native protein in order to optimize the effector immune responses that are elicited. Modified peptides are referred to herein as "analog" peptides. The term "analog" extends to any functional and/or structural equivalent of a peptide characterized by its increased stability and/or efficacy in-vivo or in-vitro in respect of the practice of the invention. The term "analog" also is used herein to extend to any amino acid derivative of the peptides as described herein.

Analogues of the peptides contemplated herein include, but are not limited to, modifications to side chains, and incorporation of unnatural amino acids and/or their derivatives, non-amino acid monomers and cross-linkers. Other methods which impose conformational constraint on the peptides or their analogs are also contemplated.

It will be apparent that the peptide of the invention can be modified in a variety of different ways without significantly affecting the functionally important immunogenic behaviour thereof. Possible modifications to the peptide sequence may include the following:

One or more individual amino acids can be substituted by amino acids having comparable or similar properties, thus:

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V may be substituted by I;
T may be substituted by S;
K may be substituted by R; and
L may be substituted by I, V or M.

5 One or more of the amino acids of peptides of the invention can be replaced by a "retro-inverso" amino acid, i.e., a bifunctional amine having a functional group corresponding to an amino acid, as discussed in WO 91/13909.

10 One or more amino acids can be deleted.

Structural analogs mimicking the 3-dimensional structure of the peptide can be used in place of the peptide itself.

15 Examples of side chain modifications contemplated by the present invention include modification of amino groups, such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH_4 ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate;
20 trinitrobenzylation of amino groups with 2, 4, 6, trinitrobenzene sulfonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction
25 with NaBH_4 .

The guanidino group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

30 The carboxyl group may be modified by carbodiimide activation via O-acylisourea formation followed by subsequent derivatization, for example, to a corresponding amide.

35 Sulfhydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of

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mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulfonic acid, phenylmercury chloride, 2-chloromercuric-4-nitrophenol and other mercurials; and carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butyglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienylalanine, and/or D-isomers of amino acids.

Crosslinkers can be used, for example, to stabilize 3-dimensional conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio (for SH) or carbodiimide (for COOH). In addition, peptides could be conformationally constrained by, for example, incorporation of α -methylamino acids, introduction of double bonds between adjacent C atoms of amino acids and

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the formation of cyclic peptides or analogs by introducing covalent bonds such as forming an amide bond between N and C termini, between two side chains or between a side chain and the N or C terminus.

5 The peptides of the invention or their analogs may occur as single length or as multiple tandem or non-tandem repeats. A single type of peptide or analog may form the repeats or the repeats may be composed of different molecules including suitable carrier molecules.

10 The immunogenicity of the peptides of the present invention may also be modulated by coupling to fatty acid moieties to produce lipidated peptides. Convenient fatty acid moieties include glycolipid analogs, N-palmityl-S-(2RS)-2,3-bis-(palmitoyloxy)propyl-cysteinyl-serine (PAM, 15 Cys-Ser), N-palmityl-S-[2,3 bis (palmitoyloxy)-(2RS)-propyl-[R]-cysteine (TPC) or a dipalmityl-lysine moiety.

 The peptides may also be conjugated to a lipidated amino acid, such as an octadecyl ester of an aromatic acid, such as tyrosine, including actadecyl-tryrosine 20 (OTH).

 Molecules in accordance with the invention may further find use in the treatment (prophylactic or curative) of AIDS and related conditions, by acting either to displace the binding of the HIV virus to human 25 or animal cells or by disturbing the 3-dimensional organization of the virus.

 A further aspect of the invention thus provides a method for the prophylaxis or treatment of AIDS or related conditions, comprising administering an effective 30 amount of a peptide in accordance with the invention.

Immunoassays

 The peptides of the present invention are useful as immunogens, as antigens in immunoassays including enzyme-linked immunosorbent assays (ELISA), RIAs and other non- 35 enzyme linked antibody binding assays, or procedures known in the art for the detection of anti-HIV

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antibodies. In ELISA assays, the peptides are immobilized onto a selected surface, for example a surface capable of binding peptides, such as the wells of a polystyrene microtitre plate. After washing to remove incompletely adsorbed peptides, a non-specific protein, such as a solution of bovine serum albumin (BSA) or casein, that is known to be antigenically neutral with regard to the test sample may be bound to the selected surface. This allows for blocking of non-specific adsorption sites on the immobilizing surface and thus decreases the background caused by non-specific bindings of antisera onto the surface.

In one diagnostic embodiment where it is desirable to identify antibodies that recognize a plurality of HIV isolates, a plurality of peptides of the present invention are immobilized onto the selected surface. Alternatively, when the B-cell epitope of a peptide of the present invention is highly conserved among various HIV isolates (for example, a B-cell epitope from gag or gp41) a single or a limited number of peptides may be immobilized. In a further diagnostic embodiment where it is desirable to specifically identify antibodies that recognize a single HIV isolate (for example, BRU, MN or SF2) a single peptide of the present invention may be immobilized. This further diagnostic embodiment has particular utility in the fields of medicine, clinical trials, law and forensic science where it may be critical to determine the particular HIV isolate that was responsible for the generation of antibodies.

Normally, the peptides are in the range of about 12 residues and up to about 14 to about 40 residues. It is understood that a mixture of peptides may be used either as an immunogen in, for example, a vaccine or as a diagnostic agent. There may be circumstances where a mixture of peptides from conserved regions and/or from the non-conserved regions are used to provide cross-

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isolate protection and/or diagnosis. In this instance, the mixture of peptide immunogens is commonly referred to as a "cocktail" preparation for use as an immunogenic composition or a diagnostic reagent.

5 The immobilizing surface is then contacted with a sample, such as clinical or biological materials to be tested, in a manner conducive to immune complex (antigen/antibody) formation. This may include diluting the sample with diluents such as solutions of BSA, bovine
10 gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to 4 hours, at temperatures such as of the order of about 25° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-
15 immunocomplexed material. The washing procedure may include washing with a solution such as PBS/Tween, or a borate buffer.

Following formation of specific immunocomplexes between the test sample and the bound peptides, and
20 subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined by subjecting the immunocomplex to a second antibody having specificity for the first antibody. If the test sample is of human origin, the second antibody is an antibody having
25 specificity for human immunoglobulins and in general IgG. To provide detecting means, the second antibody may have an associated activity, such as an enzymatic activity that will generate, for example, a colour development upon incubating with an appropriate chromogenic
30 substrate. Quantification may then be achieved by measuring the degree of colour generation using, for example, a visible spectra spectrophotometer.

Other uses

35 Molecules which bind to the conserved sequence on which the invention is based, particularly antibodies, antibody-related molecules and structural analogs

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thereof, are also of possible use as agents in the treatment and diagnosis of AIDS and related conditions.

Variants of antibodies (including an antigen binding site), such as chimeric antibodies, humanized antibodies, veneered antibodies, and engineered antibodies which bind to the peptides of the present invention are included within the scope of the invention.

Antibodies and other molecules which bind to the peptides of the present invention can be used for therapeutic (prophylactic and curative) and diagnostic purposes in a number of different ways, including the following:

For passive immunization by suitable administration of antibodies, possibly humanized antibodies, to HIV patients.

To activate, complement or mediate antibody dependent cellular cytotoxicity (ADCC) by use of antibodies of suitable subclass or isotype (possibly obtained by appropriate antibody engineering) to be capable of performing the desired function.

For targeted delivery of toxins or other agents, e.g., by use of immunotoxins comprising conjugates of antibody and a cytotoxic moiety, for binding directly or indirectly to a target conserved sequence of, for example, or gp120 or gp41.

For targeted delivery of highly immunogenic materials to the surface of HIV-infected cells, leading to possible ablation of such cells by either the humoral or cellular immune system of the host.

For detection of HIV, e.g., using a variety of immunoassay techniques.

In yet a further diagnostic embodiment, the peptide of the present invention (individually, or as mixtures including cocktail preparations) are useful for the generation of HIV antigen specific antibodies (including monoclonal antibodies) that can be used to detect HIV or

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antigens, or neutralize HIV in samples including biological samples.

In an alternative diagnostic embodiment, the peptides of the present invention can be used to specifically stimulate HIV specific T-cells in biological samples from, for example, HIV-infected individuals.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

EXAMPLES

Methods of peptide synthesis, enzyme immunoassays (EIA) and in-vitro syncytia-blocking assay (ref. 7) used by Dr. Thomas Matthews's group at Duke University (NC, USA) that are not explicitly described in this disclosure are amply reported in the scientific literature and are well within the scope of those skilled in the art.

Example I

This Example illustrates the synthesis of linear peptides.

The peptides shown in Tables I, II, VI, VII, IX, X and XI below were synthesized according to the amino acid sequences reported for the various HIV-1 isolates identified therein using the ABI (Applied Biosystems Inc) 430A peptide synthesizer and optimized t-Boc chemistry as described by the manufacturer. The crude peptides were removed from the resin by treatment with hydrofluoric acid (HF), and purified by reverse-phase high performance liquid chromatography (RP-HPLC) using a Vydac C4 semi-preparative column (1 x 30 cm) using a 15-55%

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acetonitrile gradient in 0.1% (v/v) trifluoroacetic acid (TFA) developed over 40 minutes at a flow rate of 2 ml/min. All synthetic peptides (Table I below) used in immunological testing and immunization studies were >95% pure as judged by analytical HPLC. Amino acid composition analyses performed on a Waters Pico-Tag system were in good agreement with the theoretical compositions.

Example II

10 This Example illustrates the synthesis of branched peptides.

The synthetic branched HIV-1 peptides (MAP) shown in Figure 1 were prepared using an ABI 430A peptide synthesizer and synthesized according to the method previously described by Tam (ref. 8). The MAP peptides were purified by RP-HPLC as described for the linear peptides in Example I.

Example III

20 This Example describes the protocol used to test the immunogenicity of the HIV-1 chimeric peptides.

Five 6-12 week old Balb/c (H-2^d) mice or three 6-8 week old female Duncan Hartley guinea pigs purchased from Charles River animal farm, Montreal, Canada and Hazleton animal farm, Denver, Co., USA, respectively, were individually immunized with 100 µg of the given free peptide as follows. The animals received the given dose of the peptide emulsified in Freund's complete adjuvant (CFA) or adsorbed to 3 mg of aluminium phosphate (alum) by the subcutaneous route; this was followed with a booster-dose of the same amount of the same peptide emulsified in Freund's incomplete adjuvant (IFA) or adsorbed to 3 mg of aluminium phosphate (alum) three weeks later. The mice were further boosted twice with the same amount of the same peptide prepared in the respective adjuvants at three week intervals. Sera of the experimental mice and guinea pigs collected on the

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9th and 14th day post-boosting, respectively, were assayed for peptide-specific IgG antibodies using a standard enzyme-linked immunoabsorbant assay (EIA), and assessed for syncytia-blocking activities.

5 Example IV

This Example illustrates the testing of anti-peptide antibodies using an Enzyme Immunoassay (EIA).

EIA for the detection of antibodies reactive with the V3 peptide of the different constructs was performed
10 by coating EIA plates (Covalink, Nunc, Denmark) with the respective BE- containing V3 peptides as shown in Table 1 below and Fig. 1 at 1 μ g per well according to the procedure described in reference 9. After 30 min. incubation at 4°C., the unbound peptides were removed by
15 washing the plates three times with washing buffer [phosphate-buffered saline (PBS) pH 7.0, containing 0.025% Tween 20 (Bio-rad Laboratories, Richmond, CA)]. A three-fold dilution of each of the experimental serum samples starting at 1 in 50 then was made in PBS
20 containing 0.05% skimmed milk, and 100 μ l of the diluted serum then was added to each of the peptide-coated wells. Each dilution of the serum samples was assayed in duplicate. Binding of the V3 peptide-specific antibodies to the immobilized peptide was allowed to take place by
25 incubating the plates for 1 hr at room temperature. The unbound antibodies were removed by washing the plates three times with washing buffer. One hundred microlitre of goat anti-mouse IgG antibody horse radish peroxidase conjugate (Jackson Lab.,) diluted 1 in 5,000 in washing
30 buffer as recommended by the manufacturer, then were added to each test well to detect the specific binding of the anti-V3 peptide antibody to the target peptide. After one hr of incubation at room temperature, the unbound antibody-conjugate was removed by washing the
35 plates four times with the washing buffer. The amount of bound conjugate was assayed by the addition of 100 μ l of

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a mixture of tetramethylbenzidine (TMB) and hydrogen peroxide (1 part of TMB to 9 parts of hydrogen peroxide as recommended by the manufacturer, ADI Diagnostics Inc., Willowdale, Canada). Colour development was allowed to
5 take place at room temperature in the dark for 10-15 min., and arrested by the addition of 100 μ l of 1N sulphuric acid. The optical densities of the enzyme reactions were read on a Titertek Multi Skan Spectrophotometer (MCC/340 model) at 450 nm. Results are
10 shown in Table III and are expressed as mean reciprocal reactive titres. The reciprocal titres for normal mouse sera, irrespective of the haplotypes, were always <50.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention
15 provides certain synthetic peptides comprising amino acid sequences comprising the T-cell epitopes of the HIV-1 gag protein and amino acid sequences corresponding to the V3 loop of the envelope protein including the GPGR sequence and/or the gp41 protein comprising the ELKDWA sequence,
20 tetrameric forms of such peptides, capable of eliciting an immune response to HIV-1 infection and vaccine compositions comprising such tandem synthetic peptides. Modifications are possible within the scope of this invention.

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TABLE I

HIV-1 Chimeric peptides described in this disclosure

PEPTIDE	SEQUENCE*	SEQ ID NO:
p24E	GPKEPFRDYVDRFYK	2
CLTB-36 (T-B)	GPKEPFRDYVDRFYK NKRKRIHIGPGRAFYTTK	3
CLTB-37 (B-T)	NKRKRIHIGPGRAFYTTK NGPKEPFRDYVDRFYK	4
CLTB-56 (B)	NKRKRIHIGPGRAFYTTK	5
CLTB-34 (T-B)	GPKEPFRDYVDRFYK RKRIHIGPGRAFYTTK	6
CLTB-35 (B-T)	RKRIHIGPGRAFYTTK NGPKEPFRDYVDRFYK	7
CLTB-55 (B)	RKRIHIGPGRAFYTTK	8
p24E-V3MN (T-B)	GPKEPFRDYVDRFYK RIHIGPGRAFYTTK	9
V3MN-p24E (B-T)	RIHIGPGRAFYTTK NGPKEPFRDYVDRFYK	10
V3MN (B)	RIHIGPGRAFYTTK	11
CLTB-28 (T-B)	GPKEPFRDYVDRFYK RKRIHIGPGRAF	12
CLTB-32 (B-T)	RKRIHIGPGRAF GPKEPFRDYVDRFYK	13
CLTB-29 (B)	RKRIHIGPGRAF	14
p24E-SP10 (A)	GPKEPFRDYVDRFYK CTRPNYNKRKRIHIGPGRAFYTTK	19
CLTB-91	KQIINMWQEVEKAMYANKR RKRIHIGPGRAFYTTK	23
T1-SP10 (A) MN	KQIINMWQEVEKAMYACTRPNYNKR RKRIHIGPGRAFYTTK	21
CLTB-84 (T-B)	GHKAVLAEMSVTNKR RKRIHIGPGRAFYTTK	29

* V3(MN) sequence used to construct each of the tandem epitopes in either T-B or B-T orientation is printed in bold face.

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TABLE II
Immunogenicity of HIV-1 peptide cocktail in guinea pigs

Peptide Cocktail	Sequence	Anti-peptide *		SEQ ID NO:
		IGG titre	Alum	
		Freund's		
CLTB-70 +	GPKEPFRDYVDRFYKNTKRSIYIGPGRAFHTTGR	312,500	25,000	24
CLTB-72 +	GPKEPFRDYVDRFYKNTKRIKIRIQGPGRAFTTICK	312,500	25,000	25
CLTB-74 +	GPKEPFRDYVDRFYKNTKRSITKGPGRVIYATGQ	625,000	62,500	26
CLTB-76 +	GPKEPFRDYVDRFYKNTKRSITKGLGQALYTTRG	625,000	12,500	27
p24E-GP41C	GPKEPFRDYVDRFYKSLIEESQKQKNEQELLELDKQWAS	625,000	12,500	28

* Guinea pigs were primed and boosted four times with the cocktail formulated in FA or alum. Antisera were assayed against the individually T-B tandem epitopes used to make the cocktail. Results represented the mean titre of three guinea pigs immunized with a cocktail of five different HIV-1 tandem epitopes formulated in either Freund's adjuvant or alum. The cocktail consists of: CLTB-70 (SEQ ID NO: 24), containing the V3 sequence of SF2 linked to the C-terminus of: p24E; CLTB-72 (SEQ ID NO: 25), containing the V3 sequence of IIB linked to the C-terminus of p24E; CLTB-74 (SEQ ID NO: 26), containing the V3 sequence of RF linked to the C-terminus of p24E; CLTB-76 (SEQ ID NO: 27), containing the V3 sequence of 26 linked to the C-terminus of p24E and p24E-GP41C (SEQ ID NO: 28), containing the gp41 sequence of HIV-1 (BRU) linked to the C-terminus of p24E.

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TABLE III

Immunogenicity of HIV-1 peptides

Immunizing Peptide	Reciprocal V3(MN) peptide-specific antibody titre*			
	Murine		Guinea Pig	
	Freund's	Alum	Freund's	Alum
CLTB-36 (T-B)	328,050	109,350	109,350	12,150
CLTB-37 (B-T)	12,150	1,250	12,150	450
CLTB-56 (B)	450	150	1,250	450
CLTB-34 (T-B)	109,350	12,150	109,350	12,150
CLTB-35 (B-T)	450	450	1,250	900
CLTB-55 (B)	50	50	450	150
p24E-V3MN (T-B)	36,450	1,250	36,450	2,700
V3MN-p24E (B-T)	450	450	1,250	900
V3MN (B)	<50	<50	150	150
CLTB-28 (T-B)	36,450	4,050	36,450	1,250
CLTB-32 (B-T)	150	150	12,150	450
CLTB-29 (B)	<50	<50	50	50
p24E-SP10 (A)	NC	24,300	2,700	2,700
CLTB-91	NC	145,800	58,600	8,100
CLTB-84			108,000	48,600
T1SP10 (A) -MN	300	100	900	300

* Represented as the mean reciprocal antibody titre reactive against the individual envelope BE-containing V3(MN) peptide of sera from five Balb/c (H-2d) and three Duncan Hartly Guinea Pigs immunized with the respective peptide formulated in the adjuvant indicated.

NC = not completed.

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TABLE IV

Immunogenicity of CLTB-36 in Cynomolgus Monkeys *

Monkey Number	Dose (ug)	Adjuvant	CLTB-36-specific Titre	Neutralizing Titre (MN)
14039	200	ISA 51	25,600	278
14040	200	ISA 51	12,800	430

* Monkeys were immunized intramuscularly with 200 ug of CLTB-36 emulsified in Montanide ISA 51 (Seppic) on days 0, 28 and 84. Sera collected two weeks post second boost (i.e immunization on day 84) were assayed.

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TABLE V

EIA reactivities of anti-CLTB-34 and anti-CLTB-36 antisera against V3 peptides

V3 peptide Sequence	SEQ ID NO:	Isolate	Titre			
			Anti-CLTB-34 *		Anti-CLTB-36 *	
			Murine	G.Pig	Murine	G.Pig
RKRIHIGPGRAF	31	MN	4,050	4,050	4,050	4,050
TRSIHIGPGRAF	32	SC	1,350	4,050	4,050	4,050
RRRIHIGPGRAF	33	JH3	4,050	4,050	4,050	4,050
RKSIYIGPGRAF	34	SF2	1,350	450	1,350	450
KSIRIQRGPGRAFVTIG	35	LAI	450	4,050	450	4,050
RKRIRIQRGPGRAF	36	HXB2	150	1,350	150	1,350
RKSITKGPGRVIYAT	37	RF	50	50	50	50

* Antisera were raised in Balb/c mice and guinea pigs by subcutaneous injection of 100 ug of CLTB-34 or CLTB-36 adsorbed to 1.5 mg of aluminium phosphate (alum). Results represented mean of four mouse and three guinea pig serum samples post the fourth injection.

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TABLE VI

Peptide	Sequence *	Isolate Origin of V3 sequence	SEQ ID NO:
CLTB-V3B	GPKEPFRDYVDRFYKNTRKSIRIQRGPGRAFYTIG	LAI	38
CLTB-V3RF	GPKEPFRDYVDRFYKNTRKSITKGPGRVIYATGQIIG	RF	39
CLTB-HB	GPKEPFRDYVDRFYKNTRKRRIHIGPGRVIYATGQIIG ----- MN ----- ----- RF -----	MN/RFhybrid	40
CLTB-PRI	GPKEPFRDYVDRFYKNTRKSIPIGPGRAFYTIG	Consensus of New York and Amsterdam	41
P24E-1714	GPKEPFRDYVDRFYKNTRKRRIHMGPGRAFATGDIIG	U.S. clinical isolate	42
P24E-FRE	GPKEPFRDYVDRFYKNTRKSIHIGPGRAFYTIGIIG	Consensus of French	43
CLTB-BX08	GPKEPFRDYVDRFYKNTRKSIHIGPGRAFATGELIG	French primary	44
T1-PRI	KQIINMWQEVKAMYANTRKSIPIGPGRAFYTIG	Consensus of New York and Amsterdam	45
T1-2054	KQIINMWQEVKAMYANTRKGIHIGPGRAFYTIGVDIRQ	U.S. clinical isolate	46

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TABLE VI (Cont'd)

P24M-PRI	GHKARVLAEAMSQVTNTRKRSIPIGPGRAFYTG	Consensus of New York and Amsterdam	47
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* The V3 sequence used for the construction of the individual tandem epitope peptide is bolded whereas those of the T-cell epitopes, p24E(GPKEPFRDYVDRFYK - SEQ ID NO: 2), T1 (KQIINMWQVEKAMYA - SEQ ID NO: 22) and p24M (GHKARVLAEAMSQVT - SEQ ID NO: 77) are shown in plain letters.

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TABLE VII

Peptide	Sequence *	SEQ ID NO:
CLTB-92	GPKEPFRDYVDRFYKEQELLELDKWASLNNWF	54
CLTB-92A	EQELLELDKWASLNNWF	55
CLTB-93	GPKEPFRDYVDRFYKELLELDKWASLNNWF	56
CLTB-94	ELLELDKWASLNNWF	57
CLTB-95	GPKEPFRDYVDRFYKELD KWASLNNWF	58
CLTB-96	ELDKWASLNNWF	59
CLTB-97	GPKEPFRDYVDRFYKEQELLELDKWASLNNWF	60
CLTB-97A	EQELLELDKWASLNNWF	61
LTB-98	GPKEPFRDYVDRFYKELLELD KWASLNNWF	62
CLTB-99	GPKEPFRDYVDRFYKELD KWASLNNWF	63
CLTB-100	GPKEPFRDYVDRFYKEQELLELDKWA	64
CLTB-101	GPKEPFRDYVDRFYKELLELDKWA	65
T1-KAT1	KQIINMWQVEKAMYAEQELLELDKWASLNNWF	66

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TABLE VII (Cont'd)

T1-KAT2	KQIINMWQEEVEKAMYAE ELDKWA S	67
T1-KAT3	KQIINMWQEEVEKAMYAC GPGELELELDKWA SL	68

* gp41 sequence containing Katinger's neutralization epitope (ELDKWA - SEQ ID NO: 69) used for the construction of the respective tandem epitope peptide is bolded whereas the T-cell epitopes, p24E (GPKEPFRDYVDRFYK - SEQ ID NO: 2) and T1 (KQIINMWQEEVEKAMYA - SEQ ID NO: 22) are shown in plain letters.

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TABLE VIII

Reactivity of human monoclonal antibody 2F5 against HIV-1 peptides containing the gp41 neutralization epitope

Peptide *	Absorbance (450 nm) #
CLTB-106 (negative control)	0.07
CLTB-92	0.63
CLTB-92A	0.22
CLTB-93	0.51
CLTB-94	0.34
CLTB-95	0.25
CLTB-96	0.14
CLTB-97	0.54
CLTB-98	0.58
CLTB-99	0.32
CLTB-100	0.61
CLTB-101	0.48
CLTB-102	0.65
CLTB-103	0.71
CLTB-105	0.65
CLTB-107	0.55
MPK-1	0.77
MPK-2	0.72
MPK-3	0.76
T1-KAT 1	0.54
T1-KAT 2	0.62
T1-KAT 3	0.77

* The sequences of the peptides are shown in Tables IV, VI and VIII. Each individual peptide was coated at 1 μ g per well of an ELISA plate. Human neutralizing monoclonal antibody, 2F5, was used at 40 ng per well in the ELISA protocol described in this disclosure.

Absorbance readings twice above that of the negative control (0.07) were considered as positive.

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TABLE IX

Peptide	Sequence *	Isolate Origin of V3 sequence	SEQ ID NO:
(P24N)	QMRPRGSDIAGTTSTL		70
CLTB-82	----- CLTB-56 ----- QMRPRGSDIAGTTSTLNKRKRHHIGPGRAFYTTKN	MN	71
CLTB-85	NKRKRHHIGPGRAFYTTKNQMRPRGSDIAGTTSTL	MN	72
(P24L)	EEMMTACQGVGGPGHK		73
CLTB-83	EEMMTACQGVGGPGHKNNKRKRHHIGPGRAFYTTKN	MN	74
CLTB-87	NKRKRHHIGPGRAFYTTKN EEMMTACQGVGGPGHK	MN	75
(P24M)	GHKARVLA EAMSQVT		76
CLTB-84	GHKARVLA EAMSQVTNNKRKRHHIGPGRAFYTTKN	MN	77
CLTB-89	NKRKRHHIGPGRAFYTTKN GHKARVLA EAMSQVT	MN	78
P24H	PIVQNIQQQMVHQAI		79
CLTB-156	----- PRI ----- PIVQNIQQQMVHQAINTRKRSIPIGPGRAFYTTG	Consensus of New York and Amsterdam	80
CLTB-157	NTRKRSIPIGPGRAFYTTGPIVQNIQQQMVHQAI	Consensus of New York and Amsterdam	81
T5	YKYKVVVKIEPLGVAP		82

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TABLE IX (Cont'd)

CLTB-158	YKYKVVVKIEPLGVAPNTRKSIPIGPGRAFYTTG	Consensus of New York and Amsterdam	83
CLTB-159	NTRKSIPIGPGRAFYTTGYKYKVVVKIEPLGVAP	Consensus of New York and Amsterdam	84

* The V3 sequence used for the construction of the respective tandem epitope peptide is bolded whereas the T-cell epitope is shown in plain letters.

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TABLE X

Peptide	Sequence, *	SEQ ID NO:
CLTB-102	----- gp41 ----- GPKEPFRDYVDRFYKELLELDKWASLWNWFKRRRIHIGPGRAFYTTKN ----- CLTB-56 ----- ----- gp41 -----	85
CLTB-103	----- CLTB-56 ----- GPKEPFRDYVDRFYKRRRIHIGPGRAFYTTKNELLELDKWASLWNWF ----- gp41 -----	86
CLTB-105	----- gp41 ----- ELLELDKWASLWNWFKRRRIHIGPGRAFYTTKNKGPKPFRDYVDRFYK ----- CLTB-56 -----	87
CLTB-107	----- CLTB-56 ----- NKRRIHIGPGRAFYTTKNELLELDKWASLWNWFGPKPFRDYVDRFYK ----- gp41 -----	88
T1-KAT4	----- MN-1 ----- KOIINMWQEVKAMYAKRIHIGPGRAFYTTKGPGELLELDKWASL ----- gp41 -----	89
P24E-KAT4	----- MN-1 ----- GPKEPFRDYVDRFYKRIHIGPGRAFYTTKGPGELLELDKWASL ----- gp41 -----	90
CLTB-160	----- LIP ----- GPKEPFRDYVDRFYKSIHIGPGKTLATGPGSITIGPGQVFFYRGPKRSIPIGPGRAFYTTG ----- THAI ----- ----- NYA -----	91

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TABLE X (Cont'd)

CLTB-161	----- LIP -----	THAI ----	----- NYA -----	92
	KQIINMWQVEVEKAMYAKSIHIGPKTLYATGPGSITIGPGQVFFYRGPGRKSIPIGPGRAFYTTG			

* The different V3 sequences incorporated into the respective constructs are bolded. The isolate origin of the V3 sequences are indicated. For constructs, CLTB-160 and CLTB-161, LIP denotes a consensus for the London, India and Paris isolates; THAI denotes V3 of a Thailand HIV-1 virus; and NYA denotes a consensus of the primary isolates found in New York and Amsterdam. The T-cell epitope, p24E and T1 are shown in plain letters.

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TABLE XI

Peptide	Sequence *	SEQ ID NO:
MPK-1	KQIINMWQVEVEKAMYAGPGELDKWASGPGGPKPEFRDYVDRFYK ----- T1 ----- ----- p24E -----	94
MPK-2	GPKEPFRDYVDRFYKGPGEIDKWASGPGKQIINMWQVEVEKAMYA	95
MPK-3	KQIINMWQVEVEKAMYAGPGELDKWASGPGGPKPEFRDYVDRFYK	96
MPK-4	GPKEPFRDYVDRFYKGPGEIDKWASGPGKQIINMWQVEVEKAMYA	97

* The gp41 sequence used for the construction of the respective peptide is bolded whereas the T-cell epitopes, p24E and T1 are shown in plain letters. The linker sequence GPG is italicized.

TABLE XII

Functional activity of Murine and Guinea pig antisera raised against HIV-1(MN) peptides
 Reciprocal syncytia-blocking titre a)

Antisera	Mouse		Guinea pig	
	Freund's	Alum	Freund's	Alum
CLTB-36	10	60	90	40
CLTB-37	<10	<10	10	10
CLTB-56	<10	<10	10	<10
CLTB-34	10	<10	20	40
CLTB-35	<10	<10	10	<10
CLTB-55	<10	<10	<10	<10
p24E-V3MN	80	<10	90	<10
V3MN-p24E	<10	<10	<10	<10
V3MN	<10	<10	<10	<10
CLTB-28	<10	<10	90	<10
CLTB-32	<10	<10	<10	<10
CLTB-29	<10	<10	<10	<10

a) The titres were based on >90% inhibition of syncytia formation induced by the homologous HIV-1(MN) virus.

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TABLE XIII

Immunogenicity of branched HIV-1 peptides

a) Immunizing Peptide	Reciprocal V3 (MN) peptide-specific antibody titre			
	Mouse		Guinea Pig	
	Freund's	Alum	Freund's	Alum
CLTB-36 (MAP)	12,150	12,150	24,300	12,150
CLTB-34 (MAP)	NC	NC	12,150	12,150
CLTB-91 (MAP)	NC	NC	24,300	8,100
p24E-V3MN (MAP)	NC	NC	24,300	NC
VP-TB (MAP)	NC	NC	2,700	2,700

a) Results are expressed as mean reciprocal reactive titres against the respective BE-containing peptide (depicted in Figure 1). Three guinea pigs and five mice were used for each determination.

NC: Not completed

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TABLE XIV

Functional activity of antisera raised against branched peptides

Immunizing Peptide	Reciprocal syncytia-blocking titre a)			
	Murine		Guinea Pig	
	Freund's	Alum	Freund's	Alum
CLTB-36 (MAP)	>10	>10	10	35
CLTB-34 (MAP)	NC	NC	20	40
VP-TB (MAP)	NC	NC	10	10

a) Titres were based on >90% inhibition of syncytia formation induced by the MN isolate.

NC: Not completed

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CLAIMS

What we claim is:

1. A synthetic peptide, which comprises at least one amino acid sequence comprising a T-cell epitope of the gag protein of a human immunodeficiency virus (HIV) isolate linked at the N-terminal or C-terminal end thereof, to at least one amino acid sequence comprising a B-cell epitope of the V3 loop of the envelope protein of an HIV isolate, wherein, when located at said N-terminal end, said B-cell epitope containing sequence and said T-cell epitope containing sequence are directly coupled.
2. The synthetic peptide of claim 1 wherein said HIV isolate is an HIV-1 isolate.
3. The synthetic peptide of claim 2 wherein said V3 loop is that of an HIV-1 isolate selected from the group consisting of LAV, BRU, MN, SF2, RF, PRI, 1714, 2054, HXB2, Z6, BX08, IIB and SC.
4. The synthetic peptide of claim 3 wherein said T-cell epitope containing amino acid sequence comprises one selected from P24E, P24N, P24L, P24M and P24H having the amino acid sequences shown in Tables I and IX or a portion, variation or mutant of any of the selected sequences which retains the T-cell properties of said selected sequence.
5. The synthetic peptide of claim 4 wherein said B-cell epitope containing amino acid sequence comprises the sequence GX_1GX_2 where X_1 is P or L and X_2 is R, K or Q or comprises a sequence capable of eliciting an HIV specific antiserum and recognizing the sequence GX_1GX_2 .
6. The synthetic peptide of claim 5 wherein said T-cell epitope containing amino acid sequence comprises p24E or a portion, variation or mutant thereof which retains the T-cell properties of the sequence and said B-cell epitope containing amino acid sequence comprises the sequence

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GPGR or comprises a sequence capable of eliciting HIV-specific antiserum and recognizing the sequence GPGR.

7. The synthetic peptide of claim 6 wherein said B-cell epitope containing amino acid sequence is directly coupled to the C-terminus of said T-cell containing amino acid sequence.

8. The synthetic peptide of claim 6 wherein said B-cell epitope containing amino acid sequence comprises the sequence NKRKRIHIGPGRAFYTTKN (CTLB-56) or a portion, variation or mutant thereof which retains the B-cell properties of the sequence.

9. The synthetic peptide of claim 5 wherein said B-cell containing amino acid sequence is selected from the sequences NKRKRIHIGPGRAFYTTKN (CTLB-56) RIHIGPGRAFYTTKN (V3MN), RKRIHIGPGRAF (CTLB-29), RKRIHIGPGRAFYTTKN (CTLB-55), NTRKSIYIGPGRAFHTTGR (SF2), NTRKRIRIQRGPGRAFYTTIG (LAI), NTRKSIRIQRGPGRAFYTTIG (IIIB), NTRKSITKGPRVIYATGQ (RF), NTRKSITKGPRVIYATGQIIG (RF), NTRQSTPIGLGQALYTTTRG (Z6), NTRKGIHIGPGRAFYTGEIVGDIRQ (2054), NTRKRIHMGPGRAFYATGDIIG (1714), NTRKSIHIGPGRAFYATGEIIG (BX08) or a portion, variation or mutant thereof which retains the B-cell properties of the sequence.

10. The synthetic peptide of claim 3 wherein said B-cell epitope containing sequence comprises B-cell epitope containing V3 loop sequences from at least two different HIV-1 isolates.

11. The synthetic peptide of claim 10 wherein said B-cell epitope containing amino acid sequence comprises the sequence NTRKSIRIQRGPGRAFYTTKN (VP) or NKRKRIHIGPGRVIYATGQIIG (HB), or a portion, variation or mutant thereof which retains the B-cell properties of the sequence.

12. The synthetic peptide of claim 3 wherein said B-cell epitope containing amino acid sequence comprises a consensus sequence of the V3 loop of at least two HIV-1 primary isolates.

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13. The synthetic peptide of claim 12 wherein said amino acid sequence of said B-cell epitope comprises the sequence NTRKSIPIGPGRAFYT TG (PRI), NTRKSIHIGPGRAFYT TGEIIGC (FRE) or a portion, variation or mutant thereof which retains the B-cell properties of the sequence.

14. The synthetic peptide of any one of claims 8 to 13 which is linked to the C-terminal end of said T-cell containing amino acid sequence.

15. The synthetic peptide of claim 4 wherein the B-cell epitope containing sequence is additionally linked to a further amino acid sequence containing a T-cell epitope of the gag protein or the envelope protein of HIV.

16. A synthetic peptide, which comprises at least one amino acid sequence comprising a T-cell epitope of the gag protein of a human immunodeficiency virus (HIV) isolate linked at the N-terminal or C-terminal end thereof, to at least one amino acid sequence comprising a B-cell epitope of the gp41 protein of an HIV isolate comprising the sequence $X_1\text{LKDWX}_2$, wherein X_1 is E, A, G or Q and X_2 is A or T or a sequence capable of eliciting an HIV-specific antiserum and recognizing the sequence $X_1\text{LKDWX}_2$.

17. The synthetic peptide of claim 16 wherein said HIV isolate is an HIV-1 isolate.

18. The synthetic peptide of claim 17 wherein said gp41 protein is that of an HIV-1 isolate selected from the group consisting of LAV, BRU, MN, SF2, RF, PRI, 1714, 2054, HXB2, Z6, BX08, IIIB and SC.

19. The synthetic peptide of claim 18 wherein said T-cell epitope-containing amino acid sequence comprises one selected from P24E, P24N, P24L, P24M and P24H having the amino acid sequences shown in Tables I and IX or a portion, variation or mutant of any of the selected sequence which retains the T-cell properties of said selected sequence.

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20. The synthetic peptide of claim 16 wherein said T-cell epitope containing amino acid sequence comprises p24E or a portion, variation or mutant thereof which retains the T-cell properties of the sequence and said B-cell epitope containing amino acid sequence comprises the sequence ELKDWA or comprises a sequence capable of eliciting an HIV specific antiserum and recognizing the sequence ELKDWA.

21. The synthetic peptide of claim 20 wherein said B-cell epitope containing amino acid sequence is directly coupled to the C-terminal of said T-cell containing amino acid sequence.

22. The synthetic peptide of claim 20 wherein said B-cell epitope containing amino acid sequence is selected from the sequences EQELLELDKWASLWNWFDIT (CLTB-92A), ELLELDKWASLWNWFDIT (CLTB-94), ELDKWASLWNWFDIT (CLTB-96), EQELLELDKWASLWNWF (CLTB-97A), ELLELDKWASLWNWF, ELDKWASLWNWF, EQELLELDKWA, ELLELDKWA, ELDKWAS and GPGELLELDKWASL or a portion, variation or mutant thereof which retains the B-cell properties of the sequence.

23. The synthetic peptide of claim 16 wherein said B-cell epitope containing sequence is additionally linked to an amino acid sequence comprising at least one B-cell epitope of the V3 loop of the envelope protein of an HIV isolate.

24. The synthetic peptide of claim 23 wherein said B-cell epitope is one of the sequences set forth in Table X, or a portion, variation or mutant thereof which retains the B-cell properties of the sequence.

25. The synthetic peptide of claim 19 wherein said B-cell epitope containing sequence is additionally linked to a further amino acid sequence containing a T-cell epitope of the gag protein or the envelope protein of HIV.

26. The synthetic peptide of claim 25 wherein said further B-cell epitope containing sequence is

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additionally linked to at least one further amino acid sequence containing a B-cell epitope of the gp41 or V3 loop envelope protein of an HIV isolate.

27. The synthetic peptide of claim 25 which comprises one of the amino acid sequences shown in Table XI, or a portion, variation or mutant thereof which retains the B-cell properties of the sequence.

28. A synthetic peptide molecule, comprising a plurality of individual synthetic peptides linked to form a multimeric molecule, each said individual synthetic peptides comprising an amino acid sequence comprising a T-cell epitope of a gag or envelope protein of a human immunodeficiency virus (HIV) isolate linked to an amino acid sequence comprising a B-cell epitope of a gag or envelope protein of an HIV isolate.

29. The synthetic peptide molecule of claim 28 wherein each synthetic peptide in said multimeric molecule is the same.

30. The synthetic peptide molecule of claim 28 wherein said individual synthetic peptides are selected from those claimed in claim 1 and claim 17.

31. The synthetic peptide molecule of claim 28 wherein said multimeric molecule comprises the amino acid sequence:

[GPKEPFRDYVDRFYKNKRKRIHIGPGRAFYTTKN],

or a portion, variation or mutant thereof which retains the T- and B-cell properties of the sequence.

32. The synthetic peptide molecule of claim 28 wherein said multimeric molecule comprises the amino acid sequence:

[GPKEPFRDYVDRFYKRRKRIHIGPGRAFYTTKN],

or a portion, variation or mutant thereof which retains the T- and B-cell properties of the sequence.

33. The synthetic peptide molecule of claim 28 wherein said multimeric molecule comprises the amino acid sequence:

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[GPKEPFRDYVDRFYKNTRKSIIRIQRGPGRAFYTTKN]₄,

or a portion, variation or mutant thereof which retains the T- and B-cell properties of the sequence.

34. The synthetic peptide molecule of claim 28 wherein said multimeric molecule comprises the amino acid sequence:

[KQIINWQEVEKAMYANKRKRIHIGPGRAFYTTKN]₄,

or a portion, variation or mutant thereof which retains the T- and B-cell properties of the sequence.

35. The synthetic peptide molecule of claim 28 wherein said multimeric molecule comprises the amino acid sequence:

[GPKEPFRDYVDRFYKRIHIGPGRAFYTTKN]₄,

or a portion, variation or mutant thereof which retains the T- and B-cell properties of the sequence.

36. An immunogenic composition, comprising an immunoeffective amount of at least one synthetic peptide as claimed in any one of claims 1, 16 and 28 or at least one nucleic acid molecule encoding any one of said synthetic peptides, and a pharmaceutically-acceptable carrier therefor.

37. The immunogenic composition of claim 36 comprising a plurality of ones of said synthetic peptides selected to provide an immune response to a plurality of immunologically-distinct HIV-1 isolates.

38. The immunogenic composition of claim 37 wherein said plurality of ones of said synthetic peptides are further selected to provide said immune response in a plurality of hosts differentially responsive to T-cell epitopes.

39. The immunogenic composition of claim 38 wherein said plurality of synthetic peptides comprises:

GPKEPFRDYVDRFYKNKRKRIHIGPGRAFYTTKN (CTLB-36)

KQIINWQEVEKAMYANKRKRIHIGPGRAFYTTKN (CTLB-91)

GPKEPFRDYVDRFYKNTRKSIHIGPGRAFYATGEIIG (BX08)

40. The immunogenic composition of claim 39 wherein said plurality of synthetic peptides further comprises:

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GPKEPFRDYVDRFYKGPGE LDKWASGPGKQIINMWQEVEKAMYA (MPK-2)

41. The immunogenic composition of claim 36 formulated for mucosal or parenteral administration.
42. The immunogenic composition of claim 41 further comprising at least one other immunogenic or immunostimulating material.
43. The composition of claim 42 wherein the at least one other material is an adjuvant.
44. The composition of claim 43, wherein the adjuvant is aluminum phosphate or aluminum hydroxide.
45. The composition of claim 36 formulated as a vaccine for human use.
46. A method of immunizing a host, comprising administering thereto an immunoeffective amount of the immunogenic composition of claim 36.
47. The method of claim 46, wherein the immunogenic composition is formulated for mucosal or parenteral administration.
48. The method of claim 47, wherein the immunogenic composition further comprises at least one other immunogenic or immunostimulating material.
49. The method of claim 48, wherein at least one other material is an adjuvant.
50. The method of claim 49, wherein the adjuvant is aluminum phosphate or aluminum hydroxide.
51. The method of claim 49, wherein the host is a human.
52. The method of claim 46, wherein said host is primed by at least one pre-peptide immunization with a self-assembled, non-infectious, non-replicating HIV-like particle and said administration of said immunogenic composition is effected as at least one secondary immunization of said host.
53. A diagnostic kit useful for detecting HIV specific antibodies in a test sample, the kit comprising:
 - (a) a surface;

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(b) at least one peptide having an amino acid sequence epitopically specific for the HIV-specific antibodies immobilized on the surface wherein said peptide is as claimed in any one of claims 1, 16 and 28;

(c) means for contacting the antibodies and the at least one immobilized peptide to form a complex; and

(d) means for detecting the complex.

54. A diagnostic kit for detecting HIV antigens in a test sample, the kit comprising:

(a) a surface;

(b) an antibody epitopically specific and noncross-reactive for distinct epitopes of the HIV antigen immobilized on the surface and raised to said peptide as claimed in any one of claims 1, 16 and 28;

(c) means for contacting the antibodies and the HIV antigens to form a complex; and

(d) means for detecting the complex.

55. A nucleic acid sequence coding for a synthetic peptide as claimed is any one of claims 1, 16 and 28.

56. An antibody specific for any one of the synthetic peptides claimed in any one of claims 1, 16 and 28.

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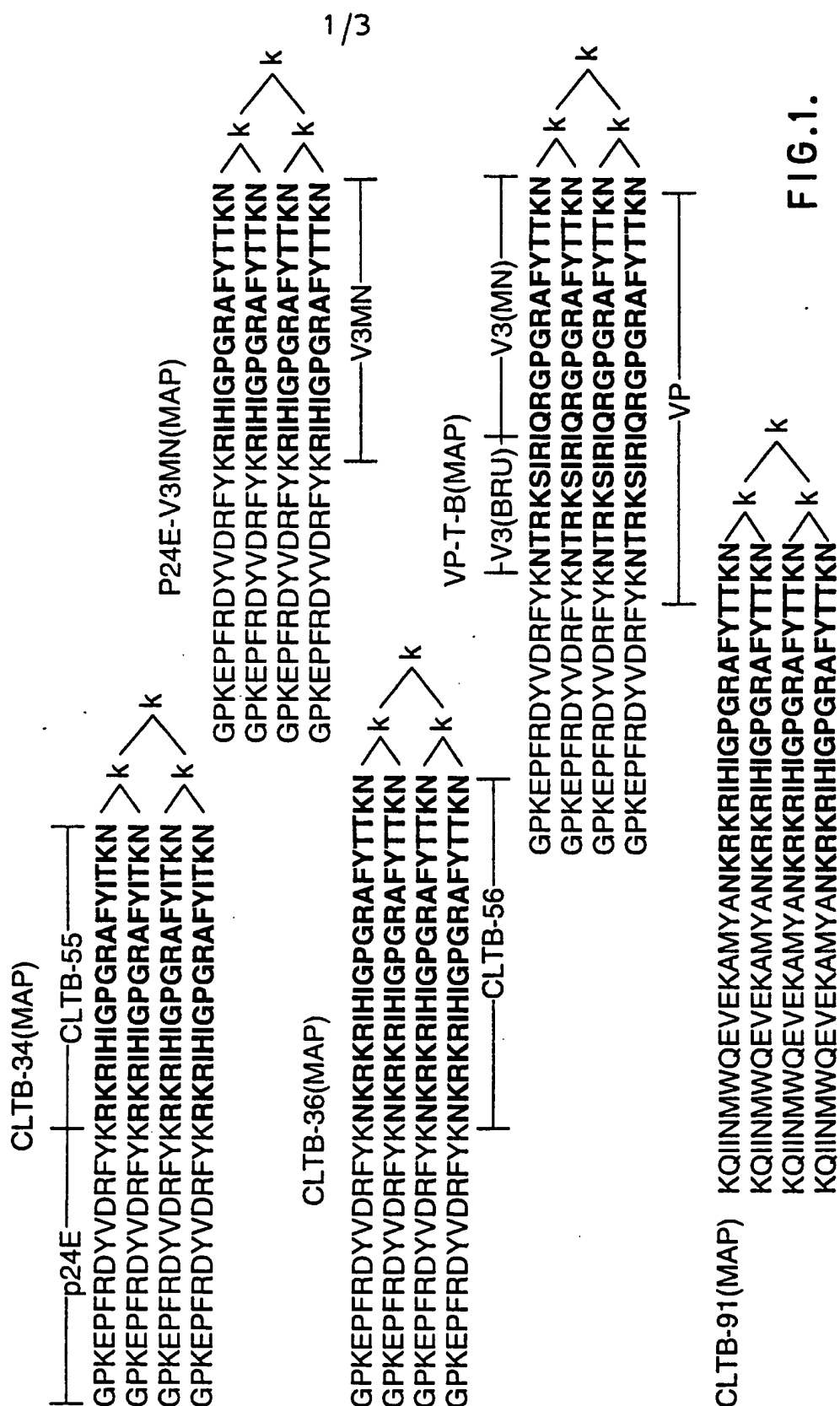


FIG.1.

2/3

Antibody responses of guinea pigs immunized with HIV-1(IIB) pseudovirion emulsified in incomplete Freund's adjuvant followed by boosting with a HIV-1 peptide cocktail formulated in alum

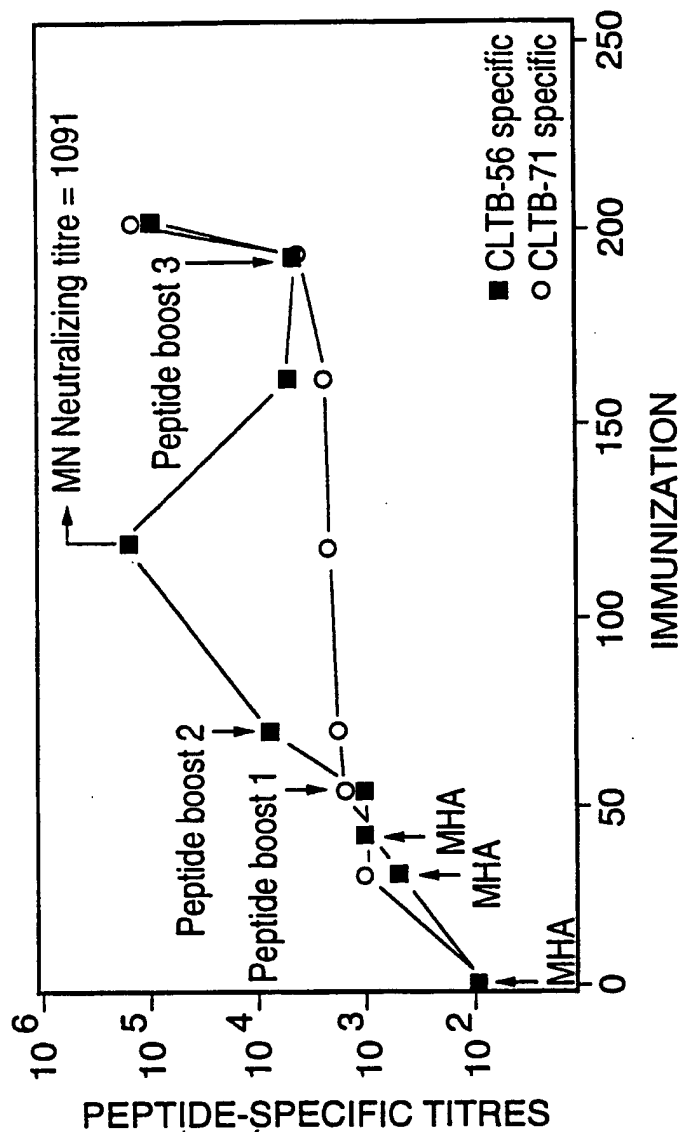
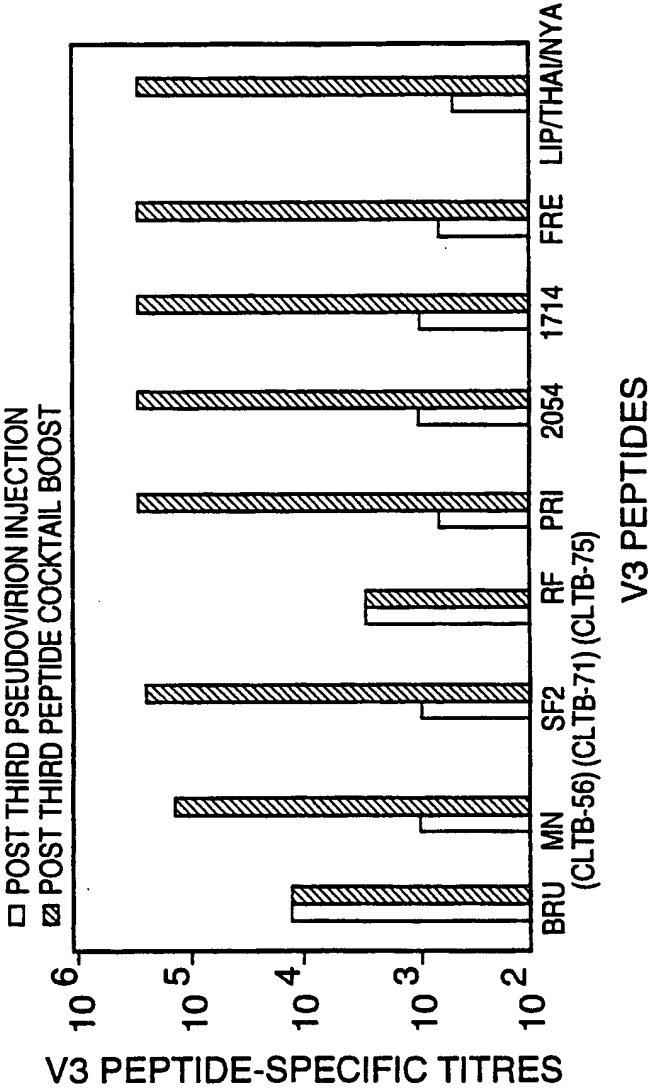


FIG.2.

Guinea pigs were injected three times with HIV-1 pseudoparticle (MHA at 40 ug equivalent of p24 per dose) emulsified in incomplete Freund's adjuvant at the times indicated. They were then boosted three times with a HIV-1 peptide cocktail consisting of 200 ug of each of the peptides: CLTB-36, CLTB-70, CLTB-91 and CLTB-84, simultaneously adsorbed to 1.5 mg of aluminium phosphate (alum) as indicated. The antibody responses generated against CLTB-56 and CLTB-71 during the course of immunization were shown. Results were expressed as mean titres of three animals.

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Reactivity of guinea pigs antisera raised against priming with HIV-1(IIB) pseudovirion emulsified in incomplete Freund's adjuvant followed by boosting with a HIV-1 peptide cocktail formulated in alum



Guinea pigs were immunized with pseudovirion (MHA) and boosted with the HIV-1 peptide cocktail as described in the footnote of Figure 2. Antisera collected after the second boost with the peptide cocktail were tested against the different V3 peptides (sequences: CLTB-56 shown in Table 1; PR1, 2054, 1714 and FRE shown in Table 4; LIP/THAI/NYA shown in Table 8; BRU, CLTB-71 and CLTB-75 shown below). Results were expressed as the mean of three guinea pigs.

BRU: NTRKSIRIQRGPGRAFTVIGKIGC
CLTB-71: NTRKSIYIGPGRAFHTTGR
CLTB-71: NTRKSITKGPGRVIYATGQ

FIG.3.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 94/00317

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C07K7/08 C07K7/10 C07K15/00 A61K39/21 C12N15/48
G01N33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,90 13564 (CONNAUGHT LABORATORIES LIMITED) 15 November 1990 cited in the application see page 4, line 27 - line 33; claims; tables 1,7 see page 7, line 33 - page 8, line 7 ---	1-7,9, 19-27, 36-38, 45,46, 53-56
X	WO,A,92 22641 (VIROGENETICS) 23 December 1992 see claims; example 50 ---	1-7,9, 19-27, 45,46, 55,56

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

Date of the actual completion of the international search

15 September 1994

Date of mailing of the international search report

26 -09- 1994

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INTERNATIONAL SEARCH REPORT

Int. Patent Application No

PCT/CA 94/00317

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
O,X	VII International Conference on AIDS Florence 16-21 June 1991 & C. Sia et al. 'Construction of Immunogenic Synthetic HIV Candidates'; Proceedings of The International Conference on AIDS, Rome, IT 1992; see Abstract M.A. 68 ---	1-7,9, 19-27, 45,46, 55,56
O,X	VIII International Conference on AIDS/ III STD World Congress Amsterdam, The Netherlands 19-24 July 1992 & C. Sia et al. 'Construction of Synthetic HIV Vaccine Candidates', Final Program & Oral Abstracts Vol. 8, No. 1, Abstract No. WeD1039 ---	1-7,9, 19-27, 45,46, 55,56
A	WO,A,91 02544 (INSTITUT PASTEUR) 7 March 1991 see claims; examples ---	1-9, 36-38, 45,46, 55,56
P,X	EP,A,0 577 894 (KOREA GREEN CROSS CORPORATION) 12 January 1994 see claims; examples -----	1-9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA94/00317

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 46-52 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 94/00317

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9013564	15-11-90	DE-D- 69007099	07-04-94
		DE-T- 69007099	01-06-94
		EP-A- 0470980	19-02-92
		JP-T- 4502013	09-04-92

WO-A-9222641	23-12-92	AU-A- 2259792	12-01-93
		EP-A- 0592546	20-04-94

WO-A-9102544	07-03-91	FR-A- 2650954	22-02-91
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		DE-T- 69008701	15-09-94
		EP-A- 0439601	07-08-91
		ES-T- 2052273	01-07-94

EP-A-0577894	12-01-94	NONE	
